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Award Number: W81XWH-05-1-0080

TITLE: The Role of C-FLIP(L) in Regulating Apoptotic Pathways in Prostate Cancer

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REPORT DATE: December 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-12-2005	Annual	15 Nov 2004 – 14 Nov 2005

4. TITLE AND SUBTITLE
The Role of C-FLIP(L) in Regulating Apoptotic Pathways in Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0080

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Beth Israel Deaconess Medical Center
Boston, MA 02215

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Abnormalities in programmed cell death (apoptosis) machinery play a crucial role in initiation, progression and metastasis of prostate cancer. Therefore, molecules that initiate pro-apoptotic pathways are excellent therapeutic agents in prostate cancer. However, some prostate cancer cells develop resistance to pro-apoptotic agents. In this proposal we are examining the regulatory mechanisms of c-FLIP(L), which is an important modulator of apoptosis in prostate cancer.

15. SUBJECT TERMS
No subject terms provided.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	7	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Progress Report

Specific Aim #1: To determine whether regulation of c-FLIP(L) in prostate cancer can affect TRAIL-sensitivity in an orthotopic prostate cancer model.

We have successfully developed our orthotopic prostate cancer model in TRAIL-sensitive and TRAIL-resistant xenografts. In this portion of our progress report, we demonstrate that TRAIL-like compounds are effective in reducing tumor burden of TRAIL-sensitive orthotopic prostate cancer xenografts. TRAIL-related therapies are currently in phase I and II clinical trials for treatment of various malignancies [1-3]. On a collaborative basis, we have obtained the TRAIL-like compound (HGS-ETR2) from Human Genome Science, Inc. (Rockville, MD) for our studies (see letter of collaboration). HGS-ETR2 activates the DR5 TRAIL receptor [1], and initiates similar extrinsic mediated caspases as TRAIL. We have found that c-FLIP(L) is also reduced in TRAIL-sensitive cells after treatment with HGS-ETR2 (data not shown), as it does after treatment with recombinant TRAIL. Therefore, HGS-ETR2 and TRAIL induce similar apoptotic pathways.

As demonstrated in our original application, some cancers are resistant to TRAIL-induced apoptosis, therefore, as part of the ongoing and future clinical trials, it is critical to be able to differentiate between patients with TRAIL-sensitive vs. TRAIL-resistant tumors. We believe that our in-vitro studies are important initial steps to identify critical molecules that can help differentiate between TRAIL-sensitive and TRAIL-resistant human tumors.

In order to determine whether our in-vitro findings can be carried over to in-vivo studies, based on our previous experience [4, 5], we designed orthotopic xenograft experiments of prostate epithelial cells. $1x10^6$ prostate epithelial cells (PC3, PC3-TR & LNCaP) were injected in the dorsa-lateral prostate of athymic male nude mice. Ten weeks after implantation, mice were treated with intravenous TRAIL-like (HGS-ETR2) compound (10 mg/kg, IV tail-vein injections, twice per week for 30 days). After completion of the 30-day treatment, mice were euthanized and analyzed for tumor size. As demonstrated in these preliminary animal results, TRAIL-like (HGS-ETR2) treatments effectively reduced the tumor burden in the TRAIL-sensitive PC3 orthotopic xenografts (Table 1, p=0.004, Mann-Whitney, two-tailed t-test), whereas, the treatment was ineffective against the TRAIL-resistant PC3-TR and LNCaP orthotopic xenografts. These results, demonstrate that TRAIL-related therapies is effective in in-vivo orthotopic models against TRAIL-sensitive PC3 xenografts and not against TRAIL-resistant PC3-TR and LNCaP xenografts. Therefore, in order to make the ongoing clinical trials more meaningful, it is critically important to be able to differentiate between patients who have TRAIL-sensitive as

	PC3		PC3-TR		LNCaP	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Avg. Tumor Weight, (mg)	887	104	120	124	3570	2250
	p=0.004		p – N.S.		p – N.S.	
# Animals	6	5	7	5	5	6
Avg. body Weight, (g)	29.4	31.4	30.9	32.8	26.8	30.7

Table 1. Pro-apoptotic treatment of orthotopically implanted prostate cancer cells. TRAIL-like (HGS-ETR2) treatment reduces the tumor burden of TRAIL-sensitive PC3 orthotopic xenografts (p=0.004, Mann-Whitney, two-tailed p value). In contrast, TRAIL-like therapy is ineffective against TRAIL-resistant PC3-TR & LNCaP cells. (N.S.= not significant)

opposed to TRAIL-resistant tumors. Currently, we are examining the expression of c-FLIP(L) in our xenografts. We are also in the process of preparing genetically modified prostate cancer cells where we can regulate the expression of c-FLIP(L) in order to assess the pro-apoptotic activity TRAIL-like compounds.

Specific Aim #2: To examine the mechanisms of transcriptional regulation of c-FLIP(L). c-Fos is differentially expressed in TRAIL-sensitive and TRAIL-resistant prostate cells. In order to investigate the genes that may differentiate between TRAIL-sensitive and TRAIL-resistant prostate epithelial cells, we used the Affymetrix gene micro-array system to compare the gene expression of cells that were treated (T) or untreated (U) with TRAIL (Table 1). We demonstrate the summary of one of our analyses, which we identified genes that were up-regulated in the TRAIL-sensitive PC3 cells after TRAIL treatment, but down-regulated in the TRAIL-resistant PC3-TR and LNCaP cells. As demonstrated in table 1, the AP-1 family member protein, c-Fos, was significantly up-regulated in the PC3 cells, but down-regulated in the PC3-TR and LNCaP cells. This finding was particularly interesting to us, because c-Fos is an important transcription factor that regulates the expression of many genes [6] and is involved in normal tissue development processes [7] and progression of many malignancies [8-11], including prostate cancer [12]. Therefore, we postulated that the c-Fos family member proteins might be an important regulator of TRAIL-induced apoptosis. We wished to determine whether c-Fos, could perhaps regulate the gene expression of c-FLIP(L) through direct or indirect interactions, leading to regulation of apoptotic pathways in prostate epithelial cells after treatment with TRAIL.

Gene	PC3 U	PC3 T	LCB	PC3-TR U	PC3-TR T	LCB	LnCap U	LnCap T	LCB
SOCS box-containing WD protein SWiP-1	91.61	155.32	1.54	210.78	102.14	-1.85	307.6	103.79	-2.7
Notch homolog 3 (Drosophila)	95.16	231.15	2.22	90.97	37.69	-1.63	126.91	49.19	-2.2
c-Fos	-0.07	53.02	**2.57**	93.08	-3.34	**-4.55**	61.81	7.13	**-2.2**
collagen, type VI, alpha 1	718.18	1790.15	2.16	235.85	136.6	-1.55	401.67	240.52	-1.5
myosin VIIB	33.06	92.81	2.53	32.59	17.05	-1.52	52.44	20.92	-1.9
hypothetical protein FLJ14360	14.36	66.69	3.79	27.88	5.79	-2.26	25.29	9.5	-1.5

Table 2. Micro-array analysis of prostate cells untreated (U) or treated (T) with TRAIL. The AP-1 family member, Fos gene, is one of the important genes that is up-regulated in the TRAIL-sensitive PC3 cells, but down-regulated in the TRAIL-resistant PC3-TR and LNCaP cells after treatment with

Putative promoter region of c-FLIP(L) gene contains multiple binding sites for c-Fos and AP-1 family member proteins. We used the GenBank and the NCBI nucleotide databases and the Tansfac 4.0 computer software program to analyze the nucleotide sequences 17,000 bp's upstream of the open reading frame of the c-FLIP(L) gene (Fig. 5). We found several binding sites for c-Fos and other AP-1 family member proteins (e.g. c-Jun, JunB, and JunD). In addition, we identified putative binding sites in the c-FLIP(L) promoter region for c-Myc and NFκB that have been shown to transcriptionally regulate the expression of c-FLIP(L) [13-15]. Therefore, since gene expression of c-Fos is differentially upregulated in the TRAIL-sensitive PC3 cells as compared to the TRAIL-resistant PC3-TR and LNCaP cells (Table 2), and there are multiple c-Fos binding sites in the putative promoter region

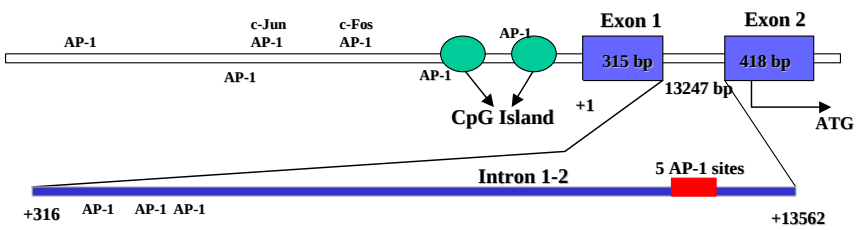

Figure 5. Putative c-FLIP(L) promoter region and the ATG start codon within exon 2. Potential binding sites for the c-Fos and other AP-1 family member proteins are shown.

of c-FLIP(L), we wished to: 1. to determine whether c-Fos has any role in mediating TRAIL-induced apoptosis in prostate epithelial cells, and 2. to determine whether c-Fos regulates transcription of c-FLIP(L) through direct and/or indirect interactions and subsequent regulation of TRAIL-induced apoptosis in prostate epithelial cells.

Specific Aim #3: To determine the expression of c-FLIP(L) in early and advanced prostate cancer. We have obtained IRB approval to use our prostate tissue cancer bank for assessment of expression of c-FLIP(L) in early and advanced prostate cancer. As a pilot project we have obtained 20 paraffin embedded samples from early to late stage prostate cancers in order to assess expression of c-FLIP(L). We plan to make more progress in this area in the coming year.

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